The perioperative care of lambs and ewes when the former undergo major experimental (scoliotic) surgery

The Perioperative Care of Lambs and Ewes When the Former Undergo Major Experimental (Scoliotic) Surgery.

Short title: Perioperative Care of Lambs & Ewes

R. Eddie Clutton[1]

Enzo Vettoratto[2]

Gudrun Schoeffman[1]

Joan Docherty[1]

John Burke[3]

J.N. Alastair Gibson[3]

[1]Royal (Dick) School of Veterinary Studies, The University of Edinburgh, Easter Bush Veterinary Centre, Roslin, Midlothian, Scotland EH25 9RG

[2]Dick White Referrals, London Road, Six Mile Bottom, Cambridgeshire

[3]The Royal Infirmary and University of Edinburgh, Little France, Edinburgh EH16 4SU

Correspondence: R. Eddie Clutton.

e-mail: e.clutton@ed.ac.uk ● telephone: 0131 650 6220 ● Fax: 0131: 650 8244

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Abstract

The purpose of the study was to optimize postoperative comfort in lambs anaesthetized for the surgical creation of scoliosis whilst maintaining the ewe-lamb relationship to miminize rejection rates. The first management plan produced 6 scoliotic lambs but intraoperative hypoventilation and hypovolaemia followed by postoperative dyspnoea, pain, monitoring and nursing difficulties, hypothermia, hypoglycaemia and tympany were encountered. Three of eight lambs (38%) were rejected by their ewes. Perioperative management was amended which, combined with improved surgical technique, produced 16 scoliotic lambs. The lambs recovered more rapidly (mean time to standing after discontinuation of anaesthesia was reduced from 12 hours to 70 minutes) and appeared to be more comfortable. No rejections occurred after the refinements were implemented.

Spinal curvatures develop in children when vertebral growth imbalances result in progressive spinal deviations in the coronal (scoliosis) sagittal (lordosis / kyphosis) and axial (rotation) planes[1,2]. Untreated children develop thoracic insufficiency with cardiorespiratory failure and ultimately death[3]. Optimal treatment involves harnessing growth[4] rather than forcibly reducing an existing deformation with instrumentation: the latter restricts pulmonary function and retards growth, preventing younger children reaching their full height. Some recently-introduced devices appear to function as growth harnesses[5] but testing their pre-commercialization performance requires a valid animal scoliotic model[6]. Despite the questionability of using quadrupeds to study disorders in bipeds, calves, pigs, and goats have been used to model vertebral column surgery for human beings. Sheep vertebra are similarly-sized to those of children and, it is believed, provide a more valid biomechanical model for studying human spinal disorders [7].

Objections to the use of lambs and kids, whose rapid post-partum growth assures their suitability as models for human skeletal growth disorders,[8] may be countered by providing optimal peri-operative care. Problems arose after the traumatic avulsion of ventral cervical spinal cord nerve roots in unweaned lambs.[9] There was a conflicting need to provide effective analgesia and surgical conditions in physiologically immature animals that had a high metabolic rate and body water content, yet low body fat, poorly developed thermoregulatory and cardiovascular reflexes and a different response to drugs. Preserving the post-operative ewe-lamb bond was also important. Anaesthetic techniques used in 8-week-old goats undergoing scoliotic surgery have been reported but details of perioperative care are very limited[10] or absent[11].

Altered attitudes to human neonatal pain management[12] have important implications in the peri-operative management of young laboratory ruminants, in which pain behaviours are less ostentatious than those of other species. This biological adaptation which serves to reduce the risk of predation probably leads to post-operative pain being under-diagnosed and under-treated.

The creation of scoliotic lambs was expected to be challenging because of the degree of surgical invasion, the anticipated duration, the fact that some animals would be younger than those previously encountered[9] and previously reported "serious" complication rates of 18%[13]. Earlier experiences also prompted reservations with the adequacy of analgesia described by McArthy et al in kids (4 IM flunixin doses [1.5 mg kg^{-1}] and a single [50 μg kg^{-1}] butorphanol injection).[10] Consequently, a more aggressive approach involving newer analgesic techniques, combined with greater emphasis on the postoperative needs of both lambs and ewes was planned. This paper describes features of perioperative management that appeared to improve the welfare of lambs and ewes involved in major experimental orthopaedic surgery.

Animals

Twenty-six Scottish Blackface ewe-lambs were purchased with their ewes from a commercial flock. Five weeks pre-purchase the ewes had been wormed and vaccinated against *Cl. perfringens* and *tetani* (Lambivac, Intervet, UK). All were in good health based on physical examination. The animals were acclimatized in purpose-designed indoor small ruminant housing within the laboratory for >10 days before the first experiment. Housing was 2 opposing lines of 5 hurdled pens (1.5 x 2m) littered with barley or wheat straw. Each lamb and its dam were confined in a single pen: water and hay were available *ad libitum* and the ewe was provided with commercial pellets (0.5 kg day^{-1} in 2 aliquots). Artificial lighting was maintained from 08:00 until 23:30 each day. The study was approved by the University's Ethical Review Committee and was licensed under the Animals (Scientific Procedures) Act 1986.

Material and Methods

Lambs were anaesthetized for the surgical creation of a right-sided scoliotic curve. Problems encountered in the first 8 lambs (including 2 fatalities) required radical technical changes. Consequently, the first 8 animals became regarded as pilot cases (and designated P(ilot) 1 – 8.

Surgery involved a dorsal midline skin incision made between T4 and L2 through which the paraspinal muscles were retracted to expose the left lamina of T4-T6 and L1-L2. A 4 mm braided synthetic Dacron tape (Abbott Spine, Austin, USA) was passed under the T5 lamina, tunnelled submuscularly, passed under the L1 lamina and looped back to form a tether. This was held under tension during the next surgical stage. Dissection bilaterally exposed the caudal six ribs. On the right, 4 cm was resected sub-periosteally from each rib avoiding breach of the thoracic pleura. On the left, the ribs were bound together with tape (3 mm

nylon, Ethicon, Ethicon UK, Livingston, UK) just distal to their angles. The combination of the rib tether and final tightening and suture of the laminar ligament was intended to produce a curvature with approximately a 25º scoliosis.

Pilot Study

Pre-anaesthetic medication was midazolam 0.5 mg kg^{-1} (Hypnovel; Roche, Welwyn Garden City, UK) and ketamine 5 mg kg^{-1} (Vetalar V 10%; Pfizer, Sandwich, UK) injected into *m. semimembranosus / semitendinosus. A*naesthesia was induced with isoflurane (IsoFlo; Abbott, Maidenhead, UK) using a Hall's pattern face mask and a Bain breathing system. Total gas flow was 3 L $minute^{-1}$. When the palpebral reflex disappeared the trachea was intubated under laryngoscopy with a suitable (5 or 6 mm cuffed) endotracheal tube (Portex Blue Line; SIMS Portex Ltd, Hythe, UK). Anaesthesia was maintained with isoflurane delivered in a 1:2 oxygen (O_2) : nitrous oxide (N_2O) mixture. The lungs were ventilated mechanically (Blease-Manley, BME, Chesham, UK) to maintain normocapnia. Neuromuscular blockade was produced with atracurium (0.5 mg kg^{-1}; Tracrium; GlaxoSmithKline, Uxbridge, UK) to improve operating conditions and suppress ventilation. Ringer's lactate solution (Vetivex 11; Dechra, Shrewsbury, UK) was infused (10 mL kg^{-1} $hour^{-1}$) and during anaesthesia, heart rate (HR) the electrocardiogram (ECG) arterial pressure (AP), end-tidal concentrations of isoflurane (F_E/ISO) and CO_2 (F_E/CO_2) and core temperature were monitored along with pulse oximetry (Datex AS/3; Datex-Engstrom, Helsinki, Finland*)*. Analgesia was intravenous (IV) meloxicam (0.6 mg kg^{-1}; Metacam; Boehringer Ingelheim, Bracknell, UK), epidural morphine (0.1 mg kg^{-1}; Morphine injection BP 1%; Martindale, Romford, UK) and, or bupivacaine (Marcaine 0.5%; AstraZeneca, Luton, UK) methylprednisolone (30 mg kg^{-1}; Solumedrone; Pfizer, Kent, UK) and buprenorphine (Vetergesic; Animalcare, York, UK) given as needed. Lambs recovered in a straw-bedded pen allowing close but limited contact with its ewe. Bottled milk replacer was offered at 3 hour intervals. The animals were observed continuously for the first 12 post-operative hours.

P1-8 had a mean age of 4.6 (range 3-6) weeks and weight of 11 (9-17) kg. Operating conditions were adequate in all cases but two lambs died. Haemorrhage occurred in P2, a lamb weighing 7 kg, which lost 208 mL (50% circulating volume) over 2 hours. Infusing 100 mls colloid (Haemaccel; Intervet, Milton Keynes, UK) failed to prevent cardiac arrest. Post-operative dyspnoea occurred in P3 in which asymmetric right thoracic and spasmodic right abdominal wall movements obscured agonal breathing patterns. *Post mortem* examination revealed total lung collapse on the left side where the tether had been over-tightened.

Other complications were: pain on midazolam / ketamine injection; difficulty in post-operative pain assessment; nursing difficulties; prolonged recoveries from post-operative hypothermia and inappetance. Intra-operative hypoventilation occurred in P1 - 5. Lambs P4, P5 and P6 were rejected by their ewes and required hand-rearing. Recovery times to first unassisted standing ranged from 12 to 20 hours, No lambs from the pilot study were re-used.

Main study

Major changes to lamb and ewe management were made after the pilot study. Forty-eight hours pre-surgery, the lambs' operation sites were clipped, sponged with surgical disinfectant (Povidone Iodine; Vetasept, Animalcare, York, UK) and covered with conforming bandage (Vetrap; 3M, Bracknell, UK) to familiarize the ewe with the lamb's post-operative appearance and smell and so reduce rejection risk. Antibiosis was begun 24 hours pre-surgery with intramuscular (IM) oxytetracycline (20 mg kg^{-1}, IM, Engemycin; Intervet, Milton Keynes, UK). Two hours pre-surgery ewes were milked to provide an alternative postoperative food option to proprietary milk replacer. Four hundred mL of maternal blood was also collected at this time and stored at room temperature (Teruflex; Terumo, Egham, UK). Lambs were allowed milk until separated from ewes for pre-anaesthetic medication. This was changed to medetomidine (10 µg kg^{-1}, Domitor; Pfizer, Sandwich, UK) injected into the epaxial muscles. The lambs were reunited with their ewes until they became recumbent.

When profound sedation and recumbency were present, anaesthesia was induced with isoflurane or sevoflurane (Sevoflo; Abbott, Maidenhead, UK) delivered in 100% oxygen. After

intubation, anaesthesia was maintained with the same agent used for induction: end-tidal isoflurane (FE′Iso) and sevoflurane (FE′Sevo) concentrations were measured and held at 1.8% and 2.8%, respectively[14]. The endotracheal tube was connected to a circle breathing system in which a Penlon Nuffield 200 Ventilator (Penlon InterMed, Abingdon, Oxon, UK) with paediatric adapter (Newton valve) was connected at the bag mount. Spirometry (Datex AS/3; Datex-Engstrom, Helsinki, Finland) revealed that tidal volumes (V_T) of 15 – 20 mL kg^{-1} delivered at a frequency (*fr*) of 17 $minute^{-1}$ produced peak inspiratory pressures of 16 – 30 cms H_2O and eucapnia, i.e., $PaCO_2$ values of 4.7 – 6.0 kPa. Atracurium was given IV as before[15].

Before surgery, meloxicam 0.6 mg kg^{-1} and morphine (0.5 mg kg^{-1}) were injected IV. A constant rate infusion of ketamine (10 μg kg^{-1} $minute^{-1}$) was delivered by syringe driver (Graseby MS16A; Smiths Medical, Ashford, UK) after a loading dose (1 mg kg^{-1}) had been given. Ketamine (0.3 mg kg^{-1}) was available in case inadequate anaesthesia or analgesia was identified. Before wound closure, bupivacaine (1.5 mg kg^{-1}) was sprayed onto the operation site using a mucosal atomization device (Wolfe Tory Medical, Waukesha, USA).

A 22 gauge cannula was placed in the auricular artery for collecting blood for gas analysis (i-STAT, i-STAT, Abbott Diagnostics, Dartford, UK) and monitoring AP. A human ear-lobe probe attached to the tongue was used for pulse oximetry (SpO_2). The oesophageal and rectal temperatures were monitored with thermistors advanced *per os* to the heart base and *per rectum* respectively. An attempt to maintain normothermia was made by increasing the ambient temperature to 22-24^{O}C. A warming pad, and a heat and moisture exchange filter (Hydro-Therm II HME, Intersurgical Ltd, Wokingham, UK) were also used.

A 22 gauge cannula was placed in either the cephalic or a lateral saphenous vein and Ringer's lactate solution infused at 10 mL kg^{-1} $hour^{-1}$. Arterial hypotension (mean AP < 55 mm Hg) was treated with ephedrine (0.1 mg kg^{-1} IV, ephedrine hydrochloride injection 3%; Martindale Pharmaceuticals, Brentwood, UK) or dextran (4 ml kg^{-1}; Dextran 40; Baxter Healthcare, Thetford, UK).

After venous access was established blood was taken and cross-matched with a sample collected earlier from the ewe using a technique described by the Animal Health Trust, UK (http://www.aht.org.uk/cms-display/diag_clinpathis1.html).

Lacrilube (Allergan Pharmaceuticals, Westport, Ireland) was placed hourly into the conjunctival sacs for corneal protection.

Haemorrhage was quantified by weighing bloodied swabs on a precision laboratory balance (PGW153e; Milton Keynes, UK). One mL blood was taken to weigh 1.3 g. This value is greater than that reported for sheep (1.08 g [16]) but was applied to compensate for evaporative plasma water losses that were likely to have occurred before measurement. The blood volume lost (Ve) through surgical suction was estimated by recording the volume in the suction jar (V), measuring *in vivo* and *in vitro* haematocrits (Hcts) and applying the equation: Ve = (*in vitro* Hct / *in vivo* Hct) X V.

Surgery was performed with lambs in the prone position supported by a vacuum-pad (Buster vacusupport; Krusse A/S, Marslev, Denmark) shaped to contour the animal's abdomen in a way that minimized intra-abdominal pressure and so reduced surgical haemorrhage. Cefuroxime (20 mg kg^{-1} IV; Zinacef; GlaxoSmithKline, Uxbridge, UK) was given immediately before surgery and continued at 8 hour intervals for 4 days. Metronidazole (20 mg kg^{-1}; Metronidazole 0.5%; Baxter Healthcare, Thetford, UK) was also given for 5 post-operative days.

When surgery ended, ketamine and inhaled anaesthetic administration were discontinued and the trachea extubated once laryngeal reflexes were forceful. The leads for physiological monitoring were left *in situ* whilst the lamb was transferred from the operating table to a sling (Fig.1). The slung lamb and multichannel monitor were then moved into a room next to the operating theatre where O_2 was available and where the lamb's ewe waited in a divided stall. The sling was positioned in the stall in a way that allowed partial contact between the two animals. The lamb's section was bare-floored but warmed with infra-red lamps and the sling was draped with bubble wrap under which a proprietary hair drier blew warm air. The ewe's section of the stall was littered with straw and equipped with food and water bowls.

Monitoring physiological variables continued in recovery along with fluid infusion. Oxygen was delivered by mask only when pulse oximetry and other signs convincingly indicated its need. Arterial samples were taken for blood-gas analysis whenever abnormal breathing patterns coincided with SpO_2 values < 0.9. Bottled ewe's milk or milk replacer was offered at hourly intervals for the first 3 hours, when venous blood samples were taken to monitor blood glucose levels (One-Touch Ultra 2; LifeScan Inc, CA, USA).

Signs of post-operative pain (plaintive bleating; bruxism, depression, disinterest in the ewe; trembling, reflex “cringing” on attempts to stand) were treated with morphine (0.5 mg kg^{-1} IV) or buprenorphine (20 μg kg^{-1} IM) and, or ketamine (0.3 mg kg^{-1} $hour^{-1}$) depending on the timing of previous doses.

Monitoring and O_2 delivery continued until the lamb could stand within the sling when the arterial cannula was removed. Fluid infusion, maintained at 10 mL kg^{-1} $hour^{-1}$ during recovery was discontinued when bottled milk was imbibed readily. At this time, the barrier between stalls was removed and the ewe allowed access to the lamb. The animals' behaviours were monitored directly for at least 20 minutes after this. At least one venous cannula was retained for the first 24 post-operative hours.

Once the lambs were standing unaided and interacting normally with the ewe they were removed from the sling, but most initially did not suck normally and required some bottled ewe’s milk or replacer. An observer was continuously present during the first hour of recovery but withdrew thereafter to monitor the animals on CCTV.

Meloxicam was injected once daily for 3 - 7 days and buprenorphine was given according to individual needs. If the latter was ineffective, IM morphine at doses up to 0.5 mg kg^{-1} were used instead. The ewe-lamb pairs were allowed into a grassed paddock from the third post-operative day if the weather was fine,

236 **Results**

237 The revised technique produced 18 scoliotic lambs, a more rapid recovery from anaesthesia[14]
238 (70 – 90 minutes to stand) and a briefer interval until successful re-unification with no lamb
239 rejection. Two lambs died however from peri-acute post-operative *Clostridium perfringens*
240 endotoxaemia[17]. The median age of animals studied was 4 weeks (range 3 - 6) weeks and
241 their body mass 12 ± 2.3 kg (means ± standard deviation [SD]).

242 Medetomidine (10 μg kg^{-1}) injection produced little reaction and profound sedation within 15
243 minutes in all lambs. Mask induction was well-tolerated and effective; tracheal intubation was
244 normally completed in 2-3 minutes[14]. The anaesthetic produced adequate surgical conditions
245 in all lambs with a median (min-max) recorded F_E'Iso of 1.8% (1.5 – 2.1). Equivalent values
246 for sevoflurane (F_E'Sevo) were 2.8% (2.5 – 3.1)[14].

247 The Penlon ventilator achieved normocapnia in all lambs without complication or modification.
248 Mean (± SD) *fr* of 17 ± 3 breaths $minute^{-1}$ and a median (min-max) peak inspiratory pressure
249 (PIP) of 22 (16-30) cms H_2O resulted in a median V_T of 16 (15 - 20) ml kg^{-1} and mean $P_E'CO_2$
250 values of 5.62 ± 0.6 kPa. When inspired O_2 concentrations were > 90% the mean (±SD)
251 arterial tension of O_2 (PaO_2) was 55.2 ± 12.4 kPa.

252 Haemodynamic variables were stable in all cases although all but one was hypotensive.
253 Ephedrine and, or dextran were effective on all occasions. After treatment for hypotension,
254 the mean intraoperative HR was 128 ± 23 beats $minute^{-1}$ while systolic, mean and diastolic
255 AP were 88 ± 11, 67 ± 12 and 56 ± 12 mm Hg, respectively.

256 Hourly arterial blood analysis revealed a mean (± SD) arterial pH of 7.43 ± 0.05, base excess
257 (BE) of 4.7±32 mmol L^{-1} and HCO_3^- of 29.0±3.2 mmol L^{-1}. Median (min-max) lactate and
258 glucose were 1.5 (0.72-2.95) and 5.75 (2.3-15.2) mmol L^{-1}, respectively.

259 No *in vitro* reactions were observed on mixing blood from 18 ewe-lamb pairs, and no adverse
260 effects were observed in the single lamb requiring transfusion (which received 25 mL kg^{-1} of
261 maternal blood at 0.5 ml kg^{-1} $hour^{-1}$ rising to 5 ml kg^{-1} $hour^{-1}$ after 30 minutes).

262 The mean (± SD) rectal temperature recorded at end-surgery was 38.69 ± $0.5^{O}C$

As the study progressed, reduced surgery times caused a corresponding reduction in anaesthesia time, with mean values of 125 ± 24 mins and 214 ± 45 mins (from induction to the discontinuation of isoflurane) respectively. No further problems were encountered with post-operative dyspnoea and post-operative blood-gas values indicated adequate pulmonary function and levels of ventilation.

Signs of post-operative pain were observed in most lambs during early recovery and were treated with variable success. The intensity and duration of pain signs diminished as surgery became briefer and less traumatic. Post-operative monitoring, fluid and O_2 administration, general nursing and maintaining normothermia were greatly facilitated by the sling (Figure 1). Lambs and ewes were re-united on average 70 minutes (range: 20 – 637) after anaesthesia was discontinued.

The animals' behaviours normalized rapidly in the first 20 minutes after re-uniting. No rejections occurred in the second group and no lambs were unable or disinclined to suck naturally. Only 1 lamb in 18 required additional morphine and no cases of tympany were observed. All lambs were behaving, suckling and moving normally within 48 hours of recovery. This process was accelerated by allowing ewe-lamb pairs access to pasture.

Modest abdominal gas distension was detected during recovery and relieved by trochar (P2) and stomach tube (P8).

Discussion

Management changes during the study's course were successful on the basis of lower morbidity, mortality, and ewe rejection rates with more rapid and more comfortable recoveries. Therefore, the changes were regarded as a refinement of methods described previously[9]. Nevertheless, two lambs unexpectedly died of Clostridial enterotoxaemia[17]. It is possible that lamb - in addition to ewe vaccination may have provided higher levels of protection after colostral immunity had waned[18] and prevented losses in the main study.

Maximizing the ewe's involvement in her offspring's peri-operative care reduces the cost of human assistance. However, this relies on the early and complete acceptance of the lamb. Any human intervention, such as that required for analgesic administration, will obviously threaten the ewe-lamb bond. In this study, effective analgesia was prioritized on practical, as well as welfare grounds; postsurgical lumbar pain could conceivably have reduced the lamb's ability to stand and "bunt" the udder to promote milk "let-down". The literature does not help solve this problem. The report of a caprine scoliosis model (McCarthy et al. 2010) limited management details to drugs and doses for anaesthesia and post-operative analgesia (*vide supra*) but provided no information on the comfort achieved and did not describe the dams' role. Other reports of scoliotic 4 – 8 week-old kids gave no information on analgesics or the role of the dam[11],[13,]

In our study, the anaesthetic provided adequate surgical conditions and uncomplicated recoveries. Medetomidine injected IM caused less reaction than the ketamine – midazolam mixture probably because ketamine causes muscle damage[19]. Ventilation was more easily controlled with the Penlon Nuffield 200. The Blease-Manley device required a "controlled leak" to restrict peak inspiratory pressures to 20 cms H_2O when gas flows and ventilator settings were producing normocapnia. The "leak" - an adjustable pressure limiting valve incorporated into the inspiratory limb - needed constant adjustment and waste-gas scavenging. Despite this, limiting lung inflation pressure to 20 cms H_2O failed to achieve normocapnia in P1 and P2, even when gas flows exceeded the minute volume of ventilation (V_M) calculated for lambs using Purves' equation[20] i.e., V_M (L minute^{-1}) = 0.123 + 0.261.x

309 (where x is body mass [kg]). Using the Penlon with a rebreathing (circle) system lowered
310 costs because gas flow and requirements were reduced. Importantly, rebreathing reduced
311 heat loss caused by breathing dry and cold gases.

312 Measures taken after P2's demise from haemorrhagic shock, i.e., contouring the surgical
313 mattress and refining surgical technique, prevented further losses from hypovolaemia. Only
314 one lamb went on to require a maternal blood transfusion. This animal lost approximately 25
315 mL kg^{-1} blood during surgery which exceeded the 15 mL kg^{-1}, that would be expected to
316 compromise circulation in a conscious adult sheep[21]. A literature review conducted after P2's
317 loss failed to confirm meloxicam's potential anticoagulant effect and so the drug was not
318 withheld from subsequent cases.

319 Haemolytic tests are preferred for grouping sheep blood, except factor D[22] (Nguyen &
320 Bunch,1980) whose antigens are detected by agglutination[23]. The lack of commercial blood-
321 typing cards or gels for sheep precluded the straightforward characterization of ewe and lamb
322 blood in the current study so transfusion reaction risks were reduced by cross-matching blood
323 of each ewe-lamb pair. No adverse *in vitro* reactions were seen in 18 cross-matches, and no
324 adverse reactions occurred in the single lamb receiving transfusion.

325 The fatal postoperative dyspnoea occurring in P3 has also been reported in caprine scoliotic
326 models with post-operative respiratory failure in 8%[13] and 7%[11] of animals. In the main part of
327 the current study, complications were avoided by more judicious tensioning of the surgical
328 tether. It would still be expected that rib resection and tethering on the convexity and
329 concavity of the curve respectively would significantly inhibit ventilatory function. In essence
330 the lamb had a flail chest wall segment on one side and limited capacity to 'over-expand' the
331 lung on the other. Although not measured the dead space would increase, possibly by as
332 much as 50%. Ventilatory function was also monitored more effectively using hourly arterial
333 blood-gas analysis in preference to continuous pulse oximetry.

334 While nursing and monitoring reunited lambs required additional staff for ewe restraint, early
335 reunification was prioritized. However, identifying the ideal time for this was complicated by
336 difficulty in interpreting the ewes' intent. This in turn was complicated by the presence of

337 people. Such difficulties contributed to three rejections during the pilot study. Lambs began
338 vocalizing in early recovery which elicited vocal responses in the ewes and prompted re-
339 unification. However, some lambs vocalized whilst recumbent, which caused two ewes to
340 vigorously "paw" their offspring in an apparent attempt to prompt standing and movement, but
341 with a force that threatened the surgical site. In contrast, the ewes of **P3** and **4** allowed
342 suckling within 2 – 3 hours of recovery from anaesthesia while **P5's** dam persistently head-
343 butted its offspring, which had to be removed and nursed manually. It became apparent
344 during the recovery of **P6** that continuous human presence contributed to ewe anxiety and
345 probably lamb rejection.

346 Measures taken to reduce maternal rejection, i.e., familiarizing ewes with their lambs' post
347 operative state, minimizing post-operative human interference using CCTV and re-uniting
348 slung lambs as soon as possible were successful because no rejections occurred in the main
349 study. That rejections were not encountered in another study using ewes of a different breed[9]
350 suggests a problem with the mothering characteristics of the Scottish Blackface. However,
351 some pilot lambs of the current study were if anything over-mothered, i.e., were vigorously
352 "pawed" by their dams.

353 The potential variation in mothering qualities within and between breeds coupled with
354 variation in the robustness of lambs complicates the choice of any specific breed for
355 experimental surgical studies. However, on the basis of the experiences described, ewe
356 familiarization, early reunification and remote postoperative surveillance are strongly
357 recommended.

358 The sling as shown in Figure 1 provided numerous benefits. Unconscious lambs could be
359 moved from the operating table whilst attached to the breathing system and physiological
360 monitors, saving time and allowing uninterrupted monitoring. By restricting lamb movement,
361 the physiological monitors, fluid and O_2 administration were resistant to disruption. The sling
362 allowed more intimate access to the lamb by the ewe whilst protecting the former from over-
363 attention. By supporting the lamb, the sling probably relieved tension in the paraspinal
364 muscles and so reduced pain both at rest and during attempts to stand that would otherwise

have been necessary. By elevating the animal from the floor, the sling assisted in keeping vascular access points clean and improved the ergonomic efficiency of nursing. The attendants did not have to repeatedly kneel and the animal and instrumentation could be moved more easily. The sling's design also contributed to restoring normothermia.

Assessing postoperative pain was difficult and there was variation in perceived comfort levels in animals receiving similar analgesics. Signs of discomfort were usually presaged by generalized shivering early in recovery. Most lambs bleated before rejoining their ewes, when they became silent (indicating that separation may have been causative) but those in discomfort continued to be depressed, reluctant to feed and disinterested in the surroundings. Bleating could be stopped by re-infusing ketamine or injecting morphine or buprenorphine, but whether this was the result of sedation or enhanced analgesia was impossible to determine. Strong, sustained bleating had the dubious benefit of demonstrating that the surgically-created flail segment was of limited functional significance.

Under non-laboratory conditions, vocal behaviours between ewes and lambs is breed-dependent and serves several purposes including location after separation[24]. Lambs also vocalize in response to acute pain caused by castration and tail docking although this does not always correlate with apparent intensity and its expression varies greatly amongst individuals[25]. However, there appears to be little information on the relative contribution of separation *versus* pain to vocalization during castration or docking which is unfortunate as such information would have been of considerable use in the current study. Until this becomes established, we propose that the absence of postoperative vocalization in separated or reunited lambs is no assurance of an absence of pain.

Other (non-vocal) signs of pain lambs related to husbandry procedures, e.g., castration, have been extensively described in lambs.[26] These, however were of limited use in the current study because the pain was different as it arose from more extensive musculoskeletal injury inflicted over a greater area.

392 Aggressive analgesia did not appear to cause problems with no signs of overdose or adverse
393 reactions observed. However, some lambs responded disappointingly to extradural drugs.
394 For example, **P7** received 2 mL (0.25 mg) bupivacaine mixed with 0.2 mg kg^{-1} morphine
395 before recovery yet looked depressed and adopted a torticollic position. Two additional
396 epidural injections (same drugs and doses) were without apparent effect. However, 6 hours
397 later the lamb made a full and rapid recovery, behaving normally thereafter. Improved
398 responsiveness of lambs to analgesics in the main, compared with the pilot study, probably
399 resulted from an improved surgical technique and associated reduction in surgery time.
400 Probably the aggressive polymodal pain therapy, with some elements given pre-emptively,
401 also helped.

402 The measures we adopted to limit hypothermia were successful in the main study. This was
403 important because the post-operative hypothermia that occurred in all the pilot lambs
404 appeared to retard recovery. Cranial nerve reflex recovery only began once re-warming
405 achieved rectal temperatures > $35^{O}C$. We observed that withholding oxygen from the
406 inspired breath in lambs with rectal temperatures between 32 and $35^{O}C$ resulted in
407 tachycardia, rapid falls in SpO_2 and parallel rises in $F_E{}^{/}CO_2$. Hypothermia probably
408 contributed to cardiac arrest in **P3** because its *ante mortem* rectal temperature was $34.4^{0}C$.
409 In the pilot study, in which lambs were unslung, attempts to achieve post-operative
410 normothermia were frustrated because the conscious animals moved away from IR heat
411 sources (and displaced the rectal thermistor probe), whilst the unmoving, sedated animals
412 were predisposed to hypothermia through inactivity. The latter presumably also had a
413 thermoregulatory reflex depression. It should be noted that the periodic recording of rectal
414 temperature using an electronic thermometer provoked signs of discomfort in some lambs
415 and anxiety in ewes. This warrants consideration being given to the use of telemetric devices
416 in future studies.

417 Finally, the variability in the ewes' readiness to suckle and the lambs' inclination to imbibe
418 complicated the maintenance of stable post-operative blood glucose levels. Our recorded
419 values ranged from 2.3 - 15.2 with a mean of 5.7 mmol L^{-1}. The published blood glucose
420 values for 4 – 8 week-old lambs range of 3.7 – 4.8 mmol L^{-1} [27] suggests that most of our were

hyperglycaemic. This perhaps was not unexpected. The postsurgical stress response in pre-term humans[28] and lambs[29] involves plasma glucose surges that parallel plasma cortisol levels and broadly reflect surgical trauma[27]. These factors prevailed against attempts to maintain normoglycaemia and so the objective re-focused on ensuring that the lambs periodically drank milk, with a rationale that this would promote sucking and ewe-lamb bonding, whilst providing glucose in the animals that were hypoglycaemic. Consequently, bottled ewes' milk, or milk replacer (approximately 180 mL) was provided at 3 hourly intervals to lambs which were not allowed or unable to suckle. Lambs refusing the bottle received 40% glucose (15 ml) IV over 10 minutes. Concerns with osmotic diuresis were dispelled by infusing crystalloid solution at supra-maintenance levels. The cause of the noted abdominal gas distension was unknown as the two animals were managed no differently from those recovering without gastro-intestinal disturbances.

Normal ewe and lamb behaviours were almost completely restored when the animals were allowed onto pasture. Under these conditions, lambs that had surgery only three days earlier displayed sucking and play behaviours that were indistinguishable in terms of complexity and vigour than those demonstrated by lambs which had not undergone surgery. Exposure to a grassy environment also appeared to accelerate the restoration of normal ewe - lamb behaviours which in some cases appeared to be subdued by indoor conditions.

In conclusion, experiments involving major surgery on lambs compromise the welfare of both the lambs and their ewes. We have outlined specific measures that will limit complications and increase the likelihood of rapid and comfortable recoveries. Ewe and possible lamb vaccination against Clostridial disease is strongly recommended

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554 **Figure 1: Legend.** A purpose-built sling for recovering lambs after major surgery. The frame
555 accommodates differently-sized subjects. Height is readily adjusted (long slider). The
556 frame's width ensures the sling is taut enough for sternal support, but does not restrict
557 breathing. Short slider adjustment allows the unconscious lamb's head to rest in a way
558 allowing single-handed tracheal intubation when necessary. The frame was 5 cm box
559 stainless steel and could be heat sterilized. The sling was washable linen toweling. All
560 measurements in cms.